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Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free β-hCG and pregnancy-associated plasma protein-A

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BACKGROUND: A beneficial consequence of screening for trisomy 21 is the early diagnosis of trisomies 18 and 13. Our objective was to examine the performance of first-trimester screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency (NT) thickness, fetal heart rate (FHR) and maternal serum-free β-hCG and pregnancy-associated plasma protein-A (PAPP-A). METHODS: Prospective screening for trisomy 21 by maternal age, fetal NT, free β-hCG and PAPP-A at 11^{+0}–13^{+6} weeks in singleton pregnancies, including 56 376 normal cases, 395 with trisomy 21, 122 with trisomy 18 and 61 with trisomy 13. Risk algorithms were developed for the calculation of patient-specific risks for each of the three trisomies based on maternal age, NT, FHR, free β-hCG and PAPP-A. Detection (DR) and false positive rates (FPR) were calculated and adjusted according to the maternal age distribution of pregnancies in England and Wales in 2000–2002. RESULTS: The DR and FPR were 90% and 3%, respectively, for trisomy 21, 91% and 0.2% for trisomy 18 and 87% and 0.2% for trisomy 13. When screen positivity was defined by an FPR of 3% on the risk for trisomy 21 in conjunction with an FPR of 0.2% on the maximum of the risks for trisomies 13 and 18, the overall FPR was 3.1% and the DRs of trisomies 21, 18 and 13 were 91%, 97% and 94%, respectively. CONCLUSIONS: As a side effect of first-trimester screening for trisomy 21, ∼95% of trisomy 13 and 18 fetuses can be detected with an 0.1% increase in the FPR.

Keywords: nuchal translucency; pregnancy-associated plasma protein-A; serum-free β-hCG; first trimester; trisomy

Introduction

Effective screening for trisomy 21 is provided by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum-free β-hCG and pregnancy-associated plasma protein-A (PAPP-A) at 11^{+0}–13^{+6} weeks of gestation with a detection rate of ∼90% for a false positive rate (FPR) of 5% (Snijders *et al*., 1998; Nicolaides *et al*., 2005). A beneficial consequence of screening for trisomy 21 is the early diagnosis of trisomies 18 and 13, which are the second and third most common chromosomal abnormalities. At 11^{+0}–13^{+6} weeks, the relative prevalence of trisomies 18 and 13 to trisomy 21 are one to three and one to seven, respectively (Snijders *et al*., 1994, 1995, 1999). All three trisomies are associated with increased maternal age, increased fetal NT and decreased maternal serum PAPP-A, but in trisomy 21 serum-free β-hCG is increased whereas in trisomies 18 and 13 this is decreased (Snijders *et al*., 1994, 1995, 1998, 1999; Tul *et al*., 1999; Spencer *et al*., 2000; Nicolaides *et al*., 2005; Wright *et al*., 2008). In addition, trisomy 13, unlike trisomies 21 and 18, is associated with fetal tachycardia (Hyett *et al*., 1996; Liao *et al*., 2000; Papageorghiou *et al*., 2006).

We have recently reported the development of a specific algorithm for trisomy 18 (Kagan *et al*., 2008a). When the algorithm for trisomy 21 (Kagan *et al*., 2008b) was used and screen positivity was fixed at an FPR of 3%, and in addition the algorithm for trisomy 18 was used and screen positivity was fixed at an FPR of 0.2%, the overall FPR was 3.1% and the detection rates of trisomies 21 and 18 were 90% and 97%, respectively (Kagan *et al*., 2008a).

The aims of this study are: first, to derive a specific algorithm for trisomy 13 based on maternal age, fetal NT thickness, fetal heart rate (FHR) and maternal serum-free β-hCG and PAPP-A; secondly, to incorporate FHR in our previously reported specific algorithms for trisomies 21 and 18 based on maternal age, fetal NT thickness and maternal serum biochemistry (Kagan *et al*., 2008a, b) and thirdly, to examine the performance of each of the three algorithms and in combination in the early detection of the three trisomies.

Methods

This was a prospective screening study for trisomy 21 in singleton pregnancies by a combination of maternal age, fetal NT thickness and maternal serum-free β-hCG and PAPP-A in a one-stop-clinic for first-trimester assessment of risk (OSCAR) at $11-13^{+6}$ weeks of gestation (Nicolaides *et al*., 2005). Transabdominal ultrasound examination was performed to diagnose any major fetal defects and for measurement of crown-rump length (CRL) and fetal NT thickness (Snijders *et al*., 1998; Nicolaides *et al*., 2005). The pregnancy was dated according to the last menstrual period, but if the dates were uncertain or the estimated gestation by CRL was discordant by more than 7 days from the estimated gestation from dates, the CRL was used to date the pregnancy. During the examination, pulsed-wave Doppler was routinely used to obtain 6–10 cardiac cycles during fetal quiescence and the FHR was calculated by the ultrasound machine software. Automated machines that provide reproducible results within 30 min were used to measure PAPP-A and free β-hCG (Bindra *et al*., 2002; Spencer *et al*., 2003; Kryptor system, Brahms AG, Berlin, Germany or Delfia Express System, Perkin Elmer, Waltham, USA).

Maternal demographic characteristics, ultrasononographic measurements and biochemical results were recorded in a computer database. Karyotype results and details on pregnancy outcomes were added into the database as soon as they became available. A search of the database was done to identify all singleton pregnancies in which first-trimester combined screening was carried out from July 1999 to April 2007.

Statistical analysis

The following steps were taken to develop a specific algorithm for the calculation of patient-specific risk of trisomy 21, trisomy 18 and trisomy 13. First, the maternal age-related risk for each trisomy at term was calculated and adjusted according to the gestational age at the time of screening (Snijders *et al*., 1994, 1995, 1999). Secondly, the measured NT was transformed into likelihood ratio for each trisomy using the mixture model of NT distributions (Wright *et al*., 2008). In both, trisomic and unaffected pregnancies fetal NT follows two distributions, one in which NT increases with CRL (CRL-dependent) and another which is CRL-independent (Fig. 1) (Wright *et al*., 2008). Thirdly, the measured free β-hCG and PAPP-A were converted into a multiples of the median (MoM) for gestational age adjusted for maternal weight, ethnicity, smoking status, method of conception, parity and machine for the assays (Kagan *et al*., 2008c). Fourthly, regression analysis was used to model mean levels of FHR in terms of gestational age, maternal age, weight, ethnicity, smoking status and method of conception. This analysis showed that deviations from the expected normal mean (delta values) were well approximated by a Gaussian distribution. Fifthly, trivariate Gaussian distributions were fitted to the joint distribution of delta FHR, log MoM free β hCG and log MoM PAPP-A in normal, trisomy 21, trisomy 18 and trisomy 13 pregnancies. Parameters associated with the distribution of log MoM free β hCG and log MoM PAPP-A were obtained from our previous studies (Kagan *et al*., 2008a, b, c). Sixthly, the likelihood ratios for NT, FHR and for the biochemical markers were multiplied with the age-related odds at the time of screening in each case. Seventhly, detection rates and FPR were calculated by taking the proportions with risks above a given risk threshold after adjustment for maternal age according to the distribution of pregnancies in England and Wales in 2000–2002 (Office for National Statistics, 2000–2002).

Results

Study population

The search of the database identified 60 172 singleton pregnancies. In 3053 (5.1%) cases, the outcome or one of the covariates was not available and in 165 (0.3%) cases there was a chromosomal abnormality other than trisomies 21, 18 or 13. Thus, our study population consisted of 56 376 pregnancies with a normal karyotype or delivery of a phenotypically normal baby (unaffected group), 395 cases of trisomy 21, 122 cases of trisomy 18 and 61 cases of trisomy 13. The characteristics of the study population are summarized in Table I.

Fetal NT thickness

The distribution of NT in fetuses with trisomies 21, 18 and 13 is shown in Fig. 1. The estimated proportions of trisomies 21, 18

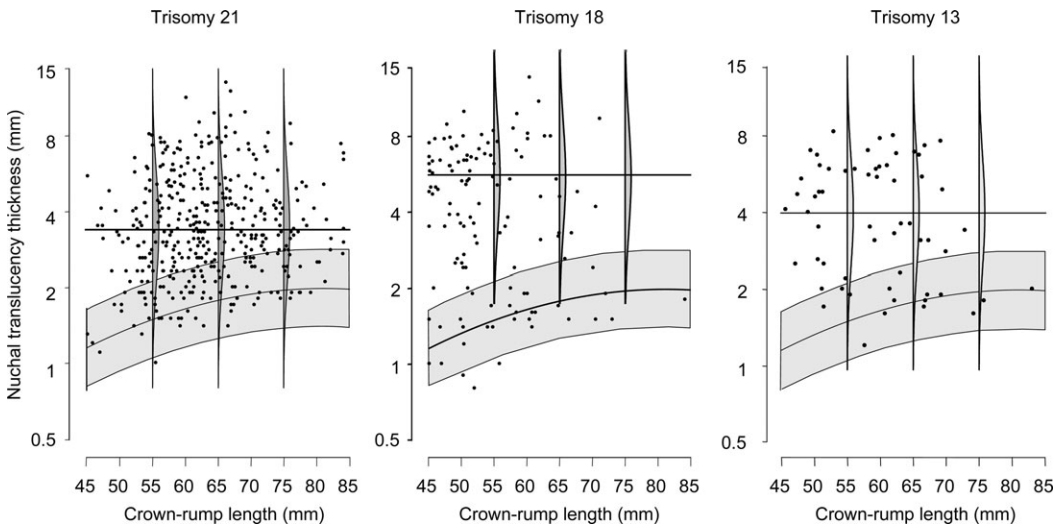

Figure 1: Distribution of fetal NT thickness with CRL in trisomies 21, 18 and 13.
In this mixture model, NT thickness was CRL-dependent (median, 5th and 95th centiles, light shaded area) in an estimated 5%, 30%, 15% and 95% of trisomies 21, 18 and 13 and unaffected fetuses, respectively, whereas in an estimated 95%, 70%, 85% and 5% of cases, respectively, NT was independent of gestation (dark shaded Gaussian distributions).

Table I. Characteristics of the study population.

Maternal characteristics	
Maternal age in years, median (range)	35.4 (14.1–52.2)
Maternal weight in kg, median (range)	63.6 (34–165)
Spontaneous conception, *n* (%)	54 306 (95.3%)
Smoker, *n* (%)	2583 (4.5%)
Ethnicity	
Caucasian, *n* (%)	50 872 (89.3%)
Afro-Caribbean, *n* (%)	2437 (4.3%)
East Asian, *n* (%)	644 (1.1%)
South Asian, *n* (%)	2224 (3.9%)
Mixed, *n* (%)	777 (1.4%)
Gestational age	
11+0–11+6 weeks, *n* (%)	5631 (9.9%)
12+0–12+6 weeks, *n* (%)	31 958 (56.1%)
13+0–13+6 weeks, *n* (%)	34.0 (34.0%)
CRL in mm, median (range)	62.8 (45.0–84.0)
Karyotype	
Normal karyotype, *n* (%)	56.376 (99.0%)
Trisomy 21, *n* (%)	395 (0.7%)
Trisomy 18, *n* (%)	122 (0.2%)
Trisomy 13, *n* (%)	61 (0.1%)
TOTAL	56 954

CRL, crown-rump length.

Table II. Ultrasonographic and biochemical characteristics of chromosomally normal fetuses and of those with trisomy 21, 18 and 13, respectively.

CRL (mm)	Median
Normal karyotype	62.8
Trisomy 21	62.4
Trisomy 18	52.0
Trisomy 13	59.4
Fetal NT (mm)	
Normal karyotype	2.0
Trisomy 21	3.4
Trisomy 18	5.5
Trisomy 13	4.0
Delta fetal heart rate (bpm)	
Normal karyotype	0
Trisomy 21	1.4
Trisomy 18	−2.8
Trisomy 13 11 weeks	20.0
Trisomy 13 12 weeks	17.2
Trisomy 13 13 weeks	14.4
PAPP-A MoM	
Normal karyotype	1.0
Trisomy 21	0.5
Trisomy 18	0.2
Trisomy 13	0.3
Free β-hCG MoM	
Normal karyotype	1.0
Trisomy 21	2.0
Trisomy 18	0.2
Trisomy 13	0.5

PAPP-A, pregnancy-associated plasma protein-A; MoM, multiples of the median; bpm, beats per minute; NT, nuchal translucency.

and 13 and unaffected fetuses that followed the CRL-independent distribution were 95%, 70%, 85% and 5%, respectively. The median CRL-independent NT was 2.0 mm for the euploid group, 3.4 mm in trisomy 21, 5.5 mm in trisomy 18 and 4.0 mm in trisomy 13 (Wright *et al.*, 2008, Table II).

Maternal serum biochemistry

Contour plots for free β-hCG and PAPP-A MoM in trisomies 21, 18 and 13 and unaffected pregnancies are shown in Fig. 2.

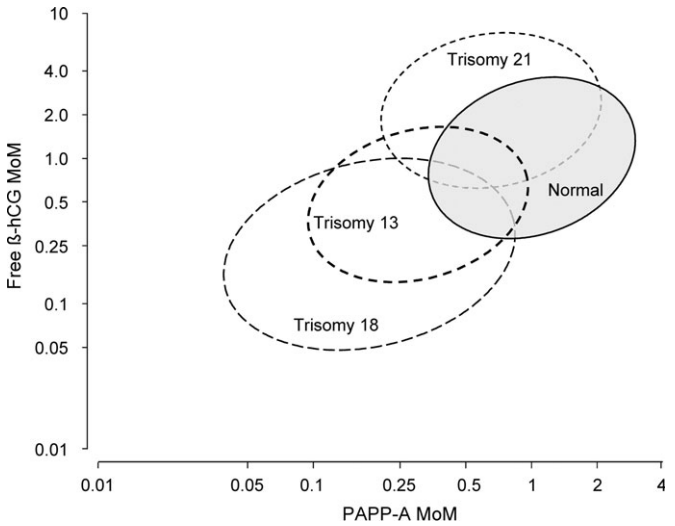

Figure 2: Distribution of MoM values of free β-hCG and of PAPP-A in normal fetuses (dark shaded ellipse), and fetuses with trisomies 21, 18 and 13 (open ellipses containing 90% of cases).

In unaffected pregnancies, the median free β-hCG was 1.0 MoM (range 0.03–30.4) and the median PAPP-A was 1.0 MoM (range 0.02–7.9), in the trisomy 21 pregnancies the median free β-hCG was 2.0 MoM (range 0.1–11.3) and the median PAPP-A was 0.5 MoM (range 0.05–2.2), in the trisomy 18 pregnancies the median free β-hCG was 0.2 MoM (range 0.02–4.7) and the median PAPP-A was 0.2 MoM (range 0.03–4.1) and in the trisomy 13 pregnancies the median free β-hCG was 0.5 MoM (range 0.19–3.3) and the median PAPP-A was 0.3 MoM (range 0.09–1.2).

Details on the biochemical parameters estimates for each chromosomal abnormality are given in Tables II and III. In trisomy 21 pregnancies, there was a significant increase with gestation in both log MoM PAPP-A ($P < 0.0001$) and log MoM free β-hCG ($P = 0.039$). In trisomy 18 pregnancies, there was no significant association with gestation for either log MoM free β-hCG ($P = 0.879$) or log MoM PAPP-A ($P = 0.900$). In trisomy 13 pregnancies, there was no significant association between log MoM MoM PAPP-A and gestation ($P = 0.38$). The association between log MoM free β-hCG and gestation was approaching significance ($P = 0.07$).

Fetal heart rate

In the multiple regression analysis of the FHR, there were significant effects of gestational age (quadratic), maternal age, ethnicity, smoking status ($P < 0.0001$) and IVF conception ($P = 0.004$) but not maternal weight ($P = 0.57$). However, for the purpose of screening, we took into account only the effect of gestation because the effects of maternal age (over a 25 year range), ethnicity, smoking status and IVF conception were <1 bpm:

Mean FHR = 265.98 – 1.7631 × gestation in days + 0.0064445 × gestation in days^2.

Parameters for the fitted Gaussian distributions are given in Table IV. There was no significant association between NT and delta FHR ($P = 0.61$). Although there were significant

Table III. Biochemical parameter estimates and correlation for trisomies 21, 18 and 13.

Parameter	Karyotype	*n*	Estimate	95% confidence limits
Mean log MoM free β-hCG	Normal	96 717	0	
	Trisomy 21—week 11		0.2596	0.2063 to 0.3129
	Trisomy 21—week 12	490	0.2895	0.2646 to 0.3143
	Trisomy 21—week 13		0.3193	0.2833 to 0.3554
	Trisomy 18	122	−0.6668	−0.7123 to −0.6213
	Trisomy 13	61	−0.3128	−0.3767 to −0.2489
Mean log MoM PAPP-A	Normal	96 717	0	
	Trisomy 21—week 11		−0.4667	−0.5256 to −0.4079
	Trisomy 21—week 12	490	−0.3026	−0.3240 to −0.2812
	Trisomy 21—week 13		−0.1385	−0.1815 to −0.0954
	Trisomy 18	122	−0.7149	−0.7549 to −0.6749
	Trisomy 13	61	−0.5248	−0.5811 to −0.4686
SD of log MoM free β-hCG	Normal	96 717	0.2544	0.2529 to 0.2559
	Trisomy 21	490	0.2699	0.2493 to 0.2940
	Trisomy 18	122	0.3723	0.3188 to 0.4454
	Trisomy 13	61	0.2416	0.1952 to 0.3140
SD of log MoM PAPP-A	Normal	96 717	0.2203	0.2190 to 0.2216
	Trisomy 21	490	0.2359	0.2179 to 0.2570
	Trisomy 18	122	0.3307	0.2832 to 0.3957
	Trisomy 13	61	0.2362	0.1908 to 0.3070
Correlation	Normal	96 717	0.2143	0.2064 to 0.2222
	Trisomy 21	490	0.0821	−0.0344 to 0.1964
	Trisomy 18	122	0.3860	0.1683 to 0.5677
	Trisomy 13	61	0.2393	−0.0939 to 0.5243

The estimates for unaffected pregnancies and for those with trisomy 21 are from our previous study (Kagan *et al*., 2008c). The means for trisomy 21 depend on gestational age according to a linear regression model. The values tabulated above are predictions for the middle of the given week.

Table IV. Distributional parameter estimates for fetal heart rate deviations from gestation specific mean.

Parameter	Karyotype	Estimate	95% confidence limits
Mean	Normal	0	
	Trisomy 21	1.3836	0.7905 to 1.9768
	Trisomy 18	−2.8089	−3.8761 to −1.7417
	Trisomy 13—week 11	20.0	17.1 to 23.0
	Trisomy 13—week 12	17.2	15.7 to 18.7
	Trisomy 13—week 13	14.4	11.5 to 17.2
SD	Normal	5.8727	5.8277 to 5.9184
	Trisomy 21	7.2323	6.6126 to 7.9699
	Trisomy 18	8.2202	7.0218 to 9.8663
	Trisomy 13	8.1444	6.5572 to 10.6360
Correlation with log MoM free β-hCG	Normal	0.0203	0.0094 to 0.0312
	Trisomy 21	0.0248	−0.1069 to 0.1557
	Trisomy 18	0.1901	−0.0487 to 0.4084
	Trisomy 13	−0.0617	−0.3851 to 0.2751
Correlation with log MoM PAPP-A	Normal	−0.0571	−0.0680 to −0.0463
	Trisomy 21	−0.0975	−0.2260 to 0.0343
	Trisomy 18	0.0624	−0.1769 to 0.2947
	Trisomy 13	−0.2378	−0.5276 to 0.1013

The means for trisomy 13 depend on gestational age according to a linear regression model. The values tabulated above are predictions for the middle of the given week.

associations between log MoM values for the biochemical markers and delta FHR ($P < 0.0001$), the magnitude of the correlations was very small (Table IV).

In trisomy 21 fetuses, the mean FHR was above the appropriate mean for gestation in unaffected pregnancies by ∼1 bpm and there was no evidence of change with gestation (Tables II and IV). In trisomy 21 fetuses, the FHR was above the 95th and 99th centiles of unaffected pregnancies in 13.7% and 6.3% of cases, respectively.

In trisomy 18 fetuses, the mean FHR was below the appropriate mean for gestation in unaffected pregnancies by ∼3 bpm and there was no evidence of change with gestation (Tables II and IV). In trisomy 18 fetuses, the FHR was below the 5th and 1st centiles of unaffected pregnancies in 17.2% and 9.0% of cases, respectively (Fig. 3).

In trisomy 13 pregnancies, there was a significant association between delta FHR and gestational age ($P = 0.02$):

Mean delta FHR in trisomy $13 = 52.43 - 0.40476 \times$ gestation in days.

In 85.2% of trisomy 13 fetuses, the FHR was above the 95th centile of unaffected pregnancies and in 62.3% cases it was above the 99th centile. The mean FHR in trisomy 13 fetuses was above the appropriate mean for gestation in unaffected pregnancies by ∼20 bpm at 11 weeks, 17 bpm at 12 weeks and 14 bpm at 13 weeks (Tables II and IV).

Performance of screening

Table V shows the detection rates for given FPR in screening for trisomies 21, 18 and 13 by each individual risk algorithm.

In screening for trisomy 21 by the risk algorithm for trisomy 21 based on maternal age and fetal NT, the detection rate was 75% for a 3% FPR. Screening by maternal age, fetal NT and serum biochemistry increased the detection rate to 89% and 90% with the addition of FHR.

In screening for trisomy 18 by the risk algorithm for trisomy 18 based on maternal age and fetal NT, the detection rate was 61% for a 0.2% FPR. Screening by maternal age, fetal NT and serum biochemistry increased the detection rate to 93% and

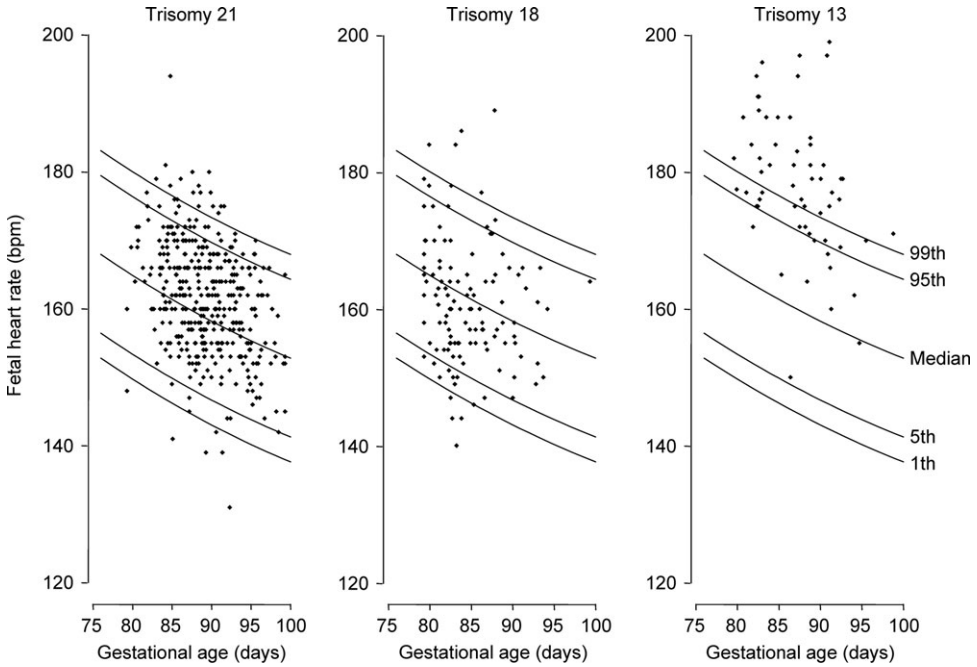

Figure 3: FHR for gestational age in trisomies 21, 18 and 13 plotted on the range of the unaffected fetuses (1st, 5th, median, 95th and 99th centile).

Table V. Detection rate of trisomies 21, 18 and 13 for given FPR in screening using the algorithm for each chromosomal abnormality.

Screening policy	Trisomy	Detection rate for fixed false positive rates (%)				
		T21 1% T13/18 0.1%	T21 2% T13/18 0.2%	T21 3% T13/18 0.3%	T21 4% T13/18 0.4%	T21 5% T13/18 0.5%
Maternal age (MA) and fetal NT	Trisomy 21	61	71	75	80	81
	Trisomy 18	56	61	65	67	68
	Trisomy 13	32	45	50	52	61
MA and serum free β-hCG and PAPP-A	Trisomy 21	40	52	58	63	67
	Trisomy 18	66	74	77	79	80
	Trisomy 13	34	42	54	57	59
MA, NT and serum free β-hCG and PAPP-A	Trisomy 21	78	84	89	90	91
	Trisomy 18	88	93	97	97	97
	Trisomy 13	66	77	84	84	84
MA, NT, free β-hCG, PAPP-A and fetal heart rate	Trisomy 21	77	84	90	91	91
	Trisomy 18	89	91	94	94	95
	Trisomy 13	87	87	87	89	89

The fixed FPR in screening for trisomy 21 (T21) are 1–5% and in screening for trisomies 13 and 18 (T13/18) the rates are 0.1–0.5%. The maternal age was adjusted according to the distribution of pregnancies in England and Wales in 2000–2002 (Office for National Statistics, 2000–2002).

Table VI. Detection rates of trisomies 21, 18 and 13 for fixed FPR using the algorithms for trisomy 21, trisomy 18 and trisomy 13, based on maternal age, fetal NT, maternal serum free β-hCG and PAPP-A and FHR.

False positive rate (%)	Detection rate (%)			False positive rate (%)	Detection rate (%)					
	Trisomy 21 algorithm				Trisomy 18 algorithm			Trisomy 13 algorithm		
	Tr 21	Tr 18	Tr 13		Tr 21	Tr 18	Tr 13	Tr 21	Tr 18	Tr 13
1	77	60	71	0.1	17	89	69	12	58	87
2	84	72	74	0.2	23	91	84	16	63	87
3	90	74	77	0.3	28	94	85	22	67	87
4	91	74	79	0.4	32	94	85	26	73	89
5	91	78	79	0.5	37	95	87	29	77	89

Table VII. Total FPR (unaffected) and detection rates of trisomies 21, 18 and 13 by the combined use of the algorithm for trisomy 21 and the algorithms for trisomies 18 and 13.

			Detection rates for fixed false positive rates using the algorithm for trisomy 21					
			0	1	2	3	4	5
Detection rates for fixed false positive rates using the algorithms for trisomies 18 and 13	0.1%	Unaffected	0.1	1.1	2.1	3.0	4.0	5.0
		Trisomy 21	17	77	85	90	91	92
		Trisomy 18	83	91	92	93	93	93
		Trisomy 13	81	88	88	90	90	90
	0.2%	Unaffected	0.2	1.2	2.1	**3.1**	4.1	5.0
		Trisomy 21	25	77	85	**91**	91	92
		Trisomy 18	94	96	96	**97**	97	97
		Trisomy 13	91	92	92	**94**	94	94
	0.3%	Unaffected	0.3	1.3	2.2	3.2	4.2	5.1
		Trisomy 21	29	77	85	90	91	92
		Trisomy 18	94	96	96	97	97	97
		Trisomy 13	91	92	92	94	94	94
	0.4%	Unaffected	0.4	1.3	2.3	3.2	4.2	5.1
		Trisomy 21	32	77	85	90	91	92
		Trisomy 18	94	96	96	97	97	97
		Trisomy 13	91	92	92	94	94	94
	0.5%	Unaffected	0.5	1.4	2.4	3.3	4.3	5.2
		Trisomy 21	36	77	85	90	91	92
		Trisomy 18	95	96	97	97	97	97
		Trisomy 13	91	92	92	94	94	94

For example, when screen positivity was defined by a 3% FPR using the algorithm for trisomy 21 and in addition by a combined 0.2% FPR using the algorithms for trisomies 18 and 13, then the detection rates of trisomies 21, 18 and 13 were 91%, 97% and 94% for a total FPR of 3.1%. The algorithms for trisomies 21,18 and 13 are based on maternal age, fetal nuchal translucency, maternal serum-free β-hCG and PAPP-A and FHR.

this was not improved by the addition of FHR (detection rate 91%).

In screening for trisomy 13 by the risk algorithm for trisomy 13 based on maternal age and fetal NT, the detection rate was 45% for a 0.2% FPR. Screening by maternal age, fetal NT and serum biochemistry increased the detection rate to 77% and this was further improved to 87% by the addition of FHR.

Table VI shows the performance of screening for trisomies 21, 18 and 13 using each of the algorithms for trisomy 21, trisomy 18 and trisomy 13, based on maternal age, fetal NT, FHR and maternal serum free β-hCG and PAPP-A. At a 3% FPR, the estimated detection rates of trisomies 21, 18 and 13 using the algorithm for trisomy 21 were 90%, 74% and 77%, respectively. The use of the algorithm for trisomy 18 identified 23%, 91% and 84% of fetuses with trisomies 21, 18 and 13, respectively, at an FPR of 0.2%. Similarly, the use of the algorithm for trisomy 13 identified 16%, 63% and 87% of fetuses with trisomies 21, 18 and 13, respectively, at an FPR of 0.2%.

Table VII shows the total FPR and detection rates of trisomies 21, 18 and 13 by the combined use of specific algorithms for trisomy 21, trisomy 18 and trisomy 13. Screen positivity was defined by a 3% FPR using the algorithm for trisomy 21 and a 0.2% FPR applied to the maximum of the risks for trisomies 18 and 13. For an overall total FPR of 3.1%, this gave detection rates for trisomies 21, 18 and 13 of 91%, 97% and 94%, respectively.

Discussion

A beneficial side effect of first-trimester combined screening for trisomy 21 is the detection of a high proportion of fetuses with trisomies 18 and 13. At a 3% FPR, the estimated detection rates of trisomies 21, 18 and 13 using the algorithm for trisomy 21 were 90%, 74% and 77%, respectively. When FHR is taken into account in screening and specific algorithms for trisomies 18 and 13 in addition to the one for trisomy 21 are also used about 90% of fetuses with trisomy 21 and 95% of those with trisomies 13 and 18 can be detected for an overall FPR of 3.1%.

In normal pregnancy, the FHR increases from ~110 bpm at 5 weeks of gestation to 170 bpm at 9 weeks and then gradually decreases to 150 bpm by 14 weeks (Liao *et al*., 2000; Wladimiroff and Seelen, 1972; Rempen *et al*., 1990; Robinson and Shaw-Dunn, 1973; Wisser and Dirschedl, 1994). The alterations in FHR observed in fetuses with trisomies 21, 18 and 13 are compatible with the results of previous studies (Hyett *et al*., 1996; Liao *et al*., 2000; Papageorghiou *et al*., 2006). Possible explanations for differences in heart rate between the various chromosomal abnormalities include differences in the types of associated cardiac defects or varying degrees of developmental delay (Hyett *et al*., 1997). The most common defects observed in trisomy 21 fetuses are atrioventricular or ventricular septal defects and relative narrowing of the aortic isthmus. In trisomy 13, there are atrioventricular or ventricular septal defects, valvular abnormalities and either narrowing of the isthmus and ascending aorta or truncus arteriosus. In trisomy 18, there are ventricular septal defects and/or polyvalvular abnormalities. Since trisomy 13 is associated with narrowing of the outflow tract from the left ventricle (Hyett *et al*., 1997), the tachycardia may be mediated by the action of baroreceptors in the aortic arch. In fetal life, the heart normally performs near the peak of the Frank–Starling curve of ventricular function (Teitel and Rudoph, 1985), and

therefore tachycardia may represent a compensatory mechanism to increase cardiac output in the phase of left heart obstruction (Rudolph and Heyman, 1976).

Inclusion of FHR in first-trimester combined sonographic and biochemical screening for chromosomal abnormalities has a small impact on the detection of trisomies 21 and 18 but a major improvement in the detection of trisomy 13. Inclusion of FHR in the risk algorithm for trisomy 13 increased the detection rate of trisomy 13 from 77% to 87% at an FPR of 0.2%. In addition, inclusion of FHR is important in distinguishing between trisomy 18 and trisomy 13, which are otherwise similar in presenting with increased fetal NT and decreased maternal serum-free β-hCG and PAPP-A.

Trisomies 18 and 13, which are the second and third most common trisomies after trisomy 21, are lethal and the rate of spontaneous abortion or fetal death between 12 weeks of gestation and 40 weeks is ∼80%. The relative prevalence of trisomy 18 to trisomy 21 and trisomy 13 to trisomy 21 at 12 weeks is one to three and one to seven, respectively, whereas at birth the respective rates are 1–12 and 1–28 (Snijders *et al*., 1995). The median survival time for children born with these disorders is 7 days and only 5% of infants survive to the end of the first year (Embleton *et al*., 1996; Rasmussen *et al*., 2003). It could therefore be argued that it is unnecessary to subject women to the difficult decisions regarding invasive testing and ultimately pregnancy termination in an affected pregnancy. The alternative view is that since many trisomy 18 and 13 fetuses can be identified during the second trimester by the presence of multiple sonographic features, women could have the option of second-trimester termination of pregnancy and avoid the risk of invasive testing if the result of first-trimester screening proves to be false positive. However, most women prefer screening to be performed early in pregnancy and in addition termination of pregnancy is safer in the first than in the second trimester (de Graaf *et al*., 2002; Bartlett *et al*., 2004). As far as the FPR is concerned, this study has demonstrated that with the addition of only 0.1% to the overall rate, ∼95% of trisomy 18 and 13 fetuses can be identified by a first-trimester screening programme for trisomy 21 with the inclusion of specific algorithms for trisomies 18 and 13. In contrast, there is no algorithm for second-trimester sonographic screening and since many of the affected fetuses present only a few of ∼20 markers, the FPR is likely to be substantially higher than 0.1%.

The National Screening Committee in the UK recommends that a screening test for trisomy 21 should provide a detection rate of at least 75% for an FPR of 3% (National Screening Committee Policy, 2006). As demonstrated in our study, for an FPR of 3.1%, first-trimester sonographic and biochemical screening by the combined use of specific algorithms for trisomies 21, 18 and 13 can detect ∼90% of fetuses with trisomy 21 and 95% of those with trisomies 13 and 18. These findings require validation from prospective studies.

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References

Bartlett LA, Berg CJ, Shulman HB, Zane SB, Green CA, Whitehead S, Atrash HK. Risk factors for legal induced abortion-related mortality in the United States. *Obstet Gynecol* 2004;**103**:729–737.

Bindra R, Heath V, Liao A, Spencer K, Nicolaides KH. One stop clinic for assessment of risk for trisomy 21 at 11–14 weeks: a prospective study of 15,030 pregnancies. *Ultrasound Obstet Gynecol* 2002;**20**:219–225.

de Graaf IM, Tijmstra T, Bleker OP, van Lith JM. Womens' preference in Down syndrome screening. *Prenat Diagn* 2002;**22**:624–629.

Embleton ND, Wyllie JP, Wright MJ, Burn J, Hunter S. Natural history of trisomy 18. *Arch Dis Child* 1996;**75**:F38–F41.

Hyett JA, Noble PL, Snijders RJ, Montenegro N, Nicolaides KH. Fetal heart rate in trisomy 21 and other chromosomal abnormalities at 10–14 weeks of gestation. *Ultrasound Obstet Gynecol* 1996;**7**:239–244.

Hyett JA, Moscoso G, Nicolaides KH. Abnormalites of the heart and great arteries in first trimester chromosomally abnormal fetuses. *Am J Med Genet* 1997;**69**:207–216.

Kagan KO, Wright D, Maiz N, Pandeva I, Nicolaides KH. Screening for trisomy 18 by maternal age, fetal NT, free β hCG and PAPP-A. *Ultrasound Obstet Gynecol* 2008a; in press.

Kagan KO, Wright D, Baker A, Sahota D, Nicolaides KH. Screening for trisomy 21 by maternal age, fetal NT, free β hCG and PAPP-A. *Ultrasound Obstet Gynecol* 2008b;**31**:618–624.

Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free beta human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. *Ultrasound Obstet Gynecol* 2008c;**31**:493–502.

Liao AW, Snijders R, Geerts L, Spencer K, Nicolaides KH. Fetal heart rate in chromosomally abnormal fetuses. *Ultrasound Obstet Gynecol* 2000; **16**:610–613.

National Screening Committee Policy. *Down's Syndrome Screening*. Compiled by the National Screening Committee. 2006. http://www.library.nhs.uk/screening.

Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol* 2005;**25**: 221–226.

Office for National Statistics. *Birth Statistics (2000–2002) Review of the Registrar General on Births and Patterns of Family Building in England and Wales*. Series FM1, Nos 29–31. London: Stationary Office.

Papageorghiou AT, Avgidou K, Spencer K, Nix B, Nicolaides KH. Sonographic screening for trisomy 13 at 11 to 13(+6) weeks of gestation. *Am J Obstet Gynecol* 2006;**194**:397–401.

Rasmussen SA, Wong LY, Yang Q, May KM, Friedman JM. Population-based analyses of mortality in trisomy 13 and trisomy 18. *Pediatrics* 2003;**111**:777–784.

Rempen A. Diagnosis of viability in early pregnancy with vaginal sonography. *J Ultrasound Med* 1990;**9**:711–716.

Robinson HP, Shaw-Dunn J. Fetal heart rates as determined by sonar in early pregnancy. *J Obstet Gynaecol Br Commonw* 1973;**90**:805–809.

Rudolph AM, Heymann MA. Cardiac output in the fetal lamb: the effects of spontaneous and induced changes of heart rate on right and left ventricular output. *Am J Obstet Gynecol* 1976;**124**:183–192.

Snijders RJM, Holzgreve W, Cuckle H, Nicolaides KH. Maternal age-specific risks for trisomies at 9–14 weeks' gestation. *Prenat Diagn* 1994;**14**:543–552.

Snijders RJM, Sebire NJ, Cuckle H, Nicolaides KH. Maternal age and gestational age-specific risks for chromosomal defects. *Fetal Diagn Ther* 1995;**10**:356–367.

Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. *Lancet* 1998;**352**:343–346.

Snijders RJM, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age and gestation-specific risk for trisomy 21. *Ultrasound Obstet Gynecol* 1999;**13**:167–170.

Spencer K, Ong C, Skentou H, Liao AW, Nicolaides KH. Screening for trisomy 13 by fetal nuchal translucency and maternal serum free beta hCG and PAPP-A at 10–14 weeks of gestation. *Prenat Diagn* 2000;**20**:411–416.

Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one stop clinic: a review of three years prospective experience. *BJOG* 2003;**110**:281–286.

Teitel D, Rudolph A. Perinatal oxygen delivery and cardiac function. *Adv Pediatr* 1985;**32**:321–347.

Tul N, Spencer K, Noble P, Chan C, Nicolaides K. Screening for trisomy 18 by fetal nuchal translucency and maternal serum free beta-hCG and PAPP-A at 10–14 weeks of gestation. *Prenat Diagn* 1999;**19**: 1035–1042.

Wisser J, Dirschedl P. Embryonic heart rate in dated human embryos. *Early Hum Dev* 1994;**37**:107–115.

Wladimiroff JW, Seelen JC. Fetal heart action in early pregnancy. Development of fetal vagal function. *Eur J Obstet Gynecol* 1972;**2**:55–63.

Wright D, Kagan KO, Molina FS, Gazzoni A, Nicolaides KH. A mixture model of nuchal translucency thickness in screening for chromosomal defects. *Ultrasound Obstet Gynecol* 2008;**31**:376–383.

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